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Official Abstract Form

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An Automatable, Colorimetric DNA Hybridization Test for *M. tuberculosis* Confirmation, BRAKEL, C.L., DONEGAN, J.J., LINN, C-I.P., MOLINA, M., POLLICE, M.A., WANG, Z., and YANG, H. L. ENZO Biochem Inc., New York, N. Y.

An oligonucleotide-based DNA hybridization test for confirmation of *M. tuberculosis* (MTB) cultures has been developed that is amenable to either partial or complete automation. Following lysis (10 min.) of cultured specimens, the hybridization is carried out in two steps and can be accomplished in less than 2 hours (20-30 minutes "hands on" time), even when as many as 30-60 specimens are to be analyzed. The lysed cultured specimens are first hybridized against one modified oligomeric probe in solution and are then allowed to hybridize to a second probe coated onto wells of microtiter (ELISA) plates. After hybridization and washing, the hybrids are detected with streptavidin-biotinylated horseradish peroxidase. Signal is generated by enzymatic conversion of hydrogen peroxide and *o*-phenylenediamine. Results can be read by eye, or quantitated with an ordinary ELISA photometer. To date the test has been 100% sensitive and specific. In a blind confirmation of 86 clinical isolates, 64 were correctly identified as MTB and 22 as non-MTB. In addition, 83 different species of bacteria, including 22 species of mycobacteria have been identified correctly as non-MTB. This methodology is suitable for the automated confirmation of any cultured organism provided suitable probes are available.

Instructions

Indicate below the subject category designation from the list on p. iv, check your poster or slide session preference, complete the check list on the reverse side of this sheet, and sign your name in the space provided.

Indicate category designation from page iv. [4-1]

Category designation

Poster/Slide Session Preference

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Please provide telephone number of signing author (212) 741-3838 x125

Area code

Slide Session 256(4) Thurs AM

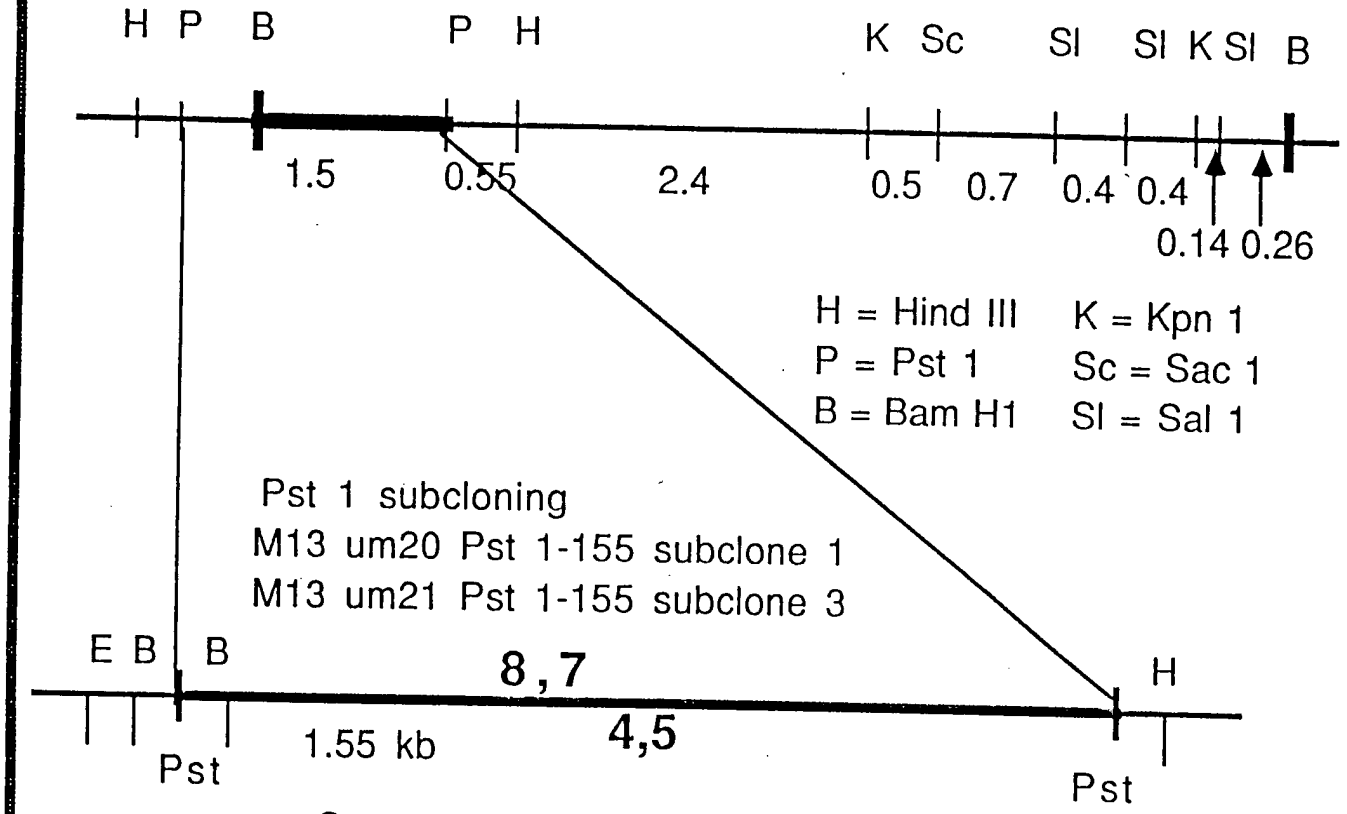
11:30 11:45

An Automatable, Colorimetric DNA Hybridization Test for M. tuberculosis Confirmation

Special Thanks to
Jim Donegan
Patsy Lin
Margarita Molina
Marjorie Pollice
Zwang Wang
Huey Lang Yang

Map and Sequences from MTB probe p24861

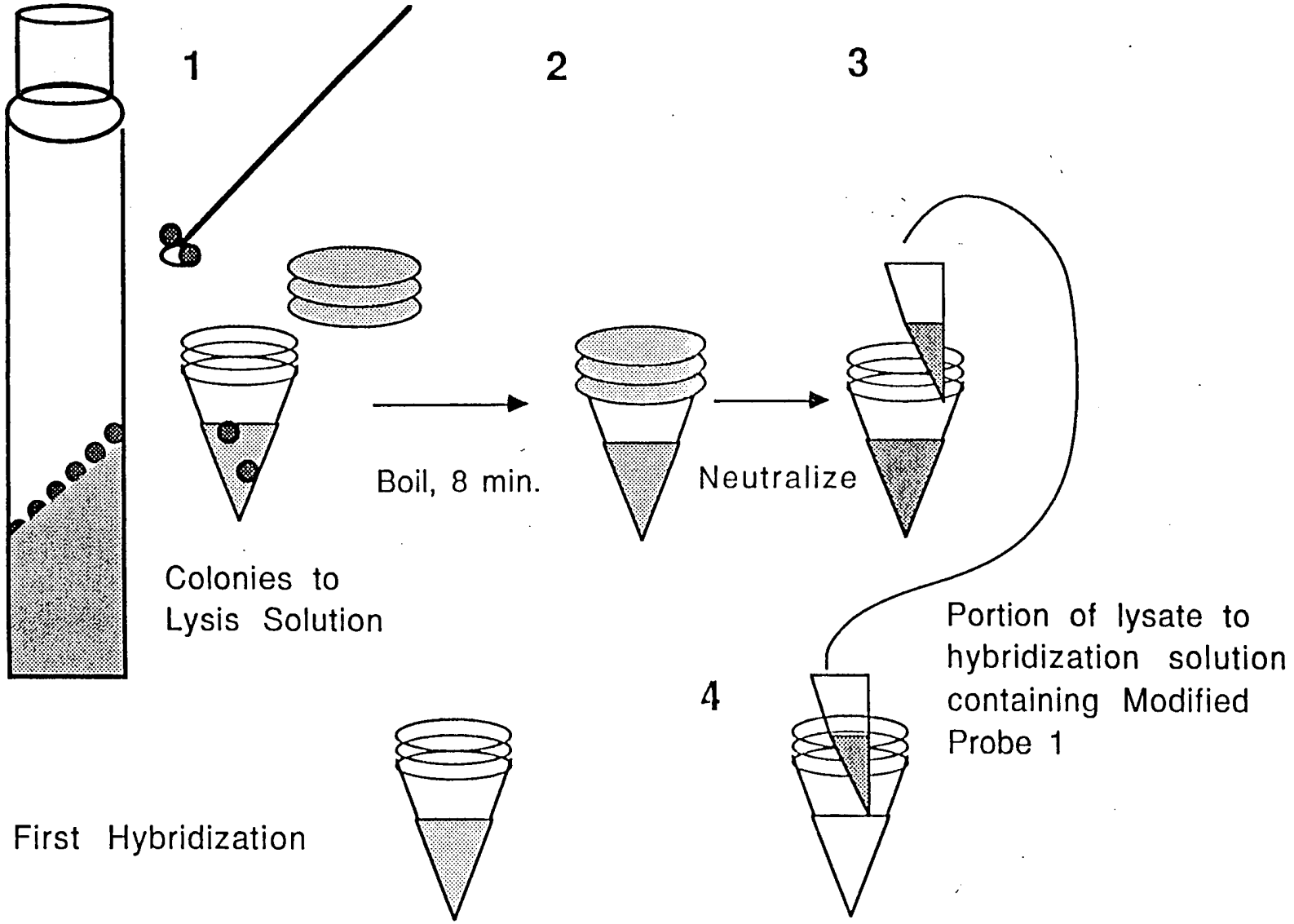
p24861 is a 7 kb insert in the Bam H1 site of pIBI 76, grown in HB101



Sequenced by Dideoxynucleotide Methods

Oligonucleotides 4, 5, 7 and 8 are 30 base sequences synthesized using an Applied Biosystems Synthesizer. The two pairs are from opposite strands of the DNA.

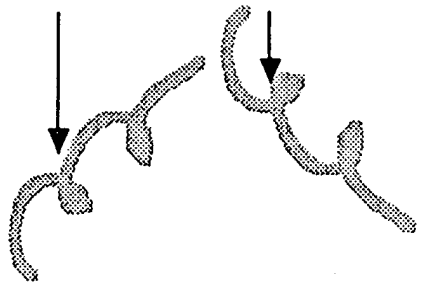
Specimen Lysis and First Hybridization



Second Hybridization and Wash

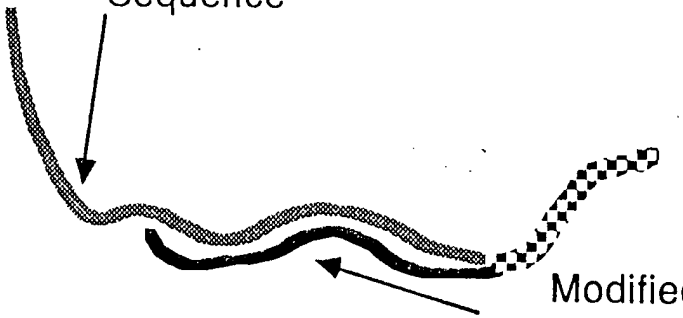
4

Non-hybridizing Sequences



Hybridizing MTB Sequences

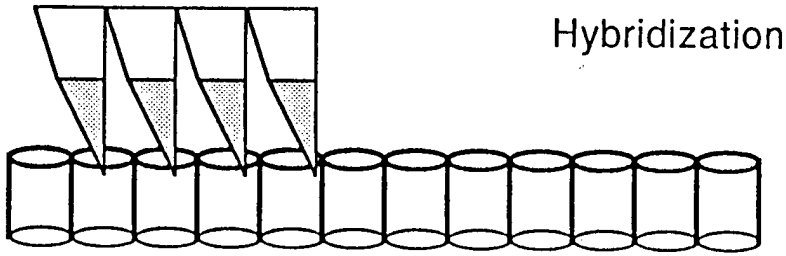
Hybridized Target Sequence



Modified Probe 1

5

Hybrid 1 added to microtiter wells containing bound Probe 2

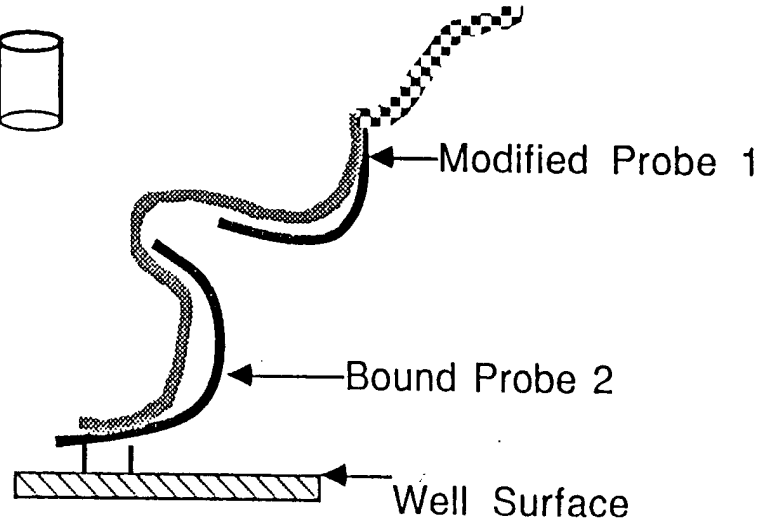


Hybridization 60 minutes minimum

6

Wash to remove non-hybridizing DNA

Only MTB-specific DNA remains



Detection of Hybridized MTB DNA

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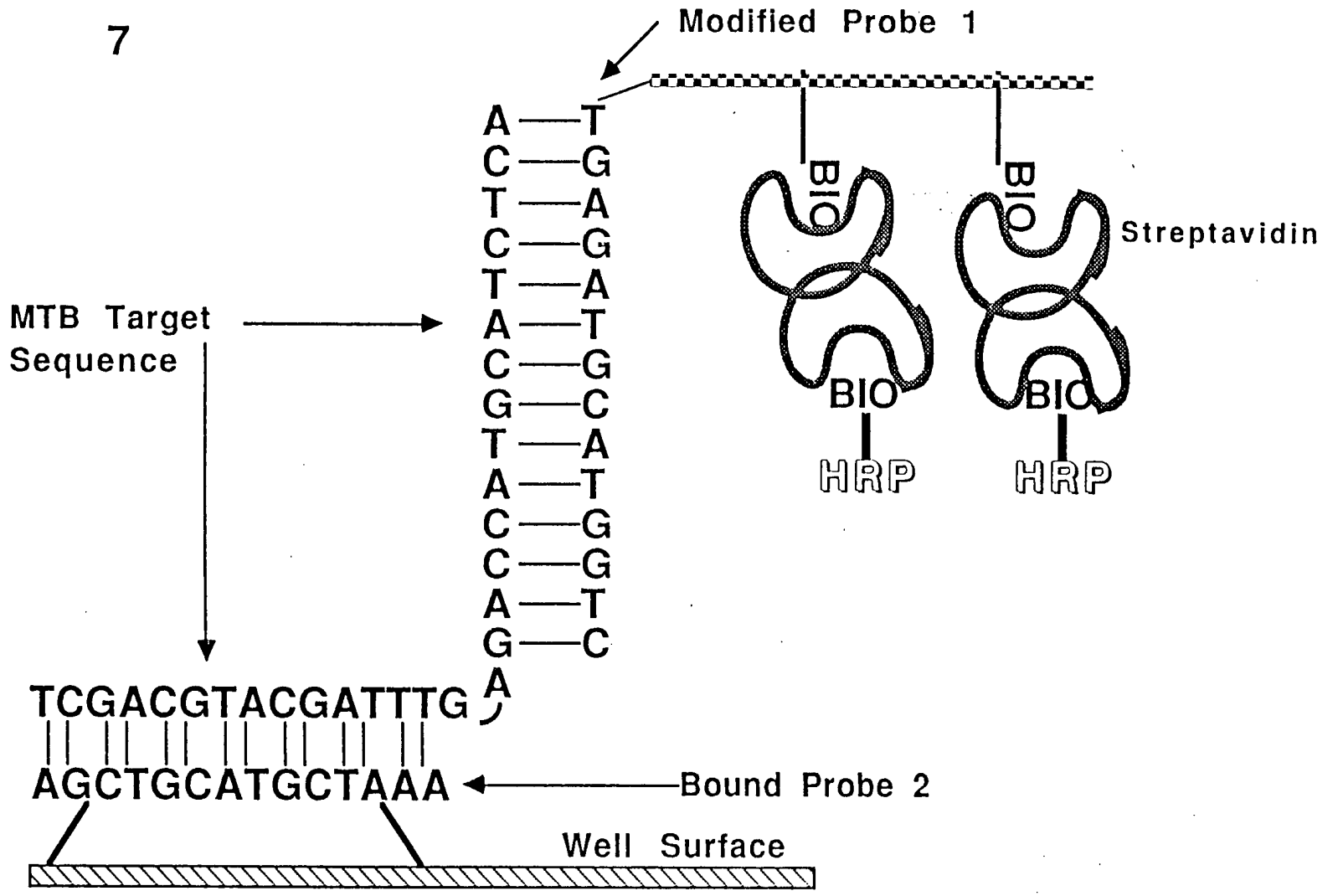

(NOTE: Sequences are examples only, and are not the actual sequences used.)

Cross Reaction Studies

Acinetobacter calcoaceticus
A. lwoffii
Actinomadura madurae
Actinoplanes italicus
Arthrobacter oxydans
Bacillus subtilis
Bacterionema matruchotii
Bacteroides fragilis
Branhamella Catarrhalis
Brevibacterium linens
Campylobacter jejuni
Chromobacterium violaceum
Clostridium perfringens
Corynebacterium aquaticum
C. diphtheriae
C. genitalium
C. haemolyticum
C. minutissimum
C. pseudodiphtheriticum
C. pseudogenitalium
C. pseudotuberculosis
C. pyogenes
C. renale
C. striatum
C. xerosis
Deinococcus radiodurans
Dermatophilus congolensis
Derxia gummosa
Enterobacter aerogenes
Escherichia coli
Fusobacterium nucleatum
Haemophilus influenzae
Klebsiella pneumoniae
Legionella pneumophila
Microbacterium lacticum
Mycoplasma hominis
M. pneumoniae
Neisseria gonorrhea
N. lactamica
N. meningitidis
Nocardia asteroides
N. brasiliensis
N. otitidis-caviarum
Nocardiopsis dassonvillei
Oerskovia turbata
O. xanthineolytica
Propionibacterium acnes
Proteus mirabilis
Pseudomonas aeruginosa
P. cepacia
Rahnella aquatilis
Rhodococcus aichiensis
R. aurantiacus
R. bronchialis
R. chubeuensis
R. equi
R. sputi
Rhodospirillum rubrum
Staphylococcus aurea
Streptococcus mitis
S. pneumoniae
Vibrio parahaemolyticus
Yersinia enterocolitica

63 Bacterial Species found to be Not Cross Reactive

Cross Reaction Studies Mycobacterial Species

POSITIVE REACTIONS

MTB Complex
Mycobacterium africanum
Mycobacterium bovis
Mycobacterium bovis BCG
Mycobacterium tuberculosis
Mycobacterium microti

Positive for MTB Complex
Negative for 25 other
Mycobacterial Species

NEGATIVE REACTIONS

M. asiaticum
M. avium
M. chelonae
M. flavenscens
M. fortuitum
M. gastri
M. gordonae
M. haemophilum
M. intracelluare
M. kansasii
M. malmoense
M. marinum
M. nonchromogenicum
M. phlei
M. scrofulaceum
M. shimoidei
M. simiae
M. smegmatis
M. szulgai
M. terrae
M. thermoresistible
M. triviale
M. ulcerans
M. vaccae
M. xenopi

Results of Testing

Study 1

DNA Assay	Culture +	Culture -
+	72	1 *
-	1 * *	49

*Originally identified as M. xenopi. Later found to contain MTB and Corynebacterium pseudotuberculosis.

**Originally identified as MTB. Later found to be Brevibacterium linens.

Study 2

DNA Assay	Culture/GenProbe +	Culture/GenProbe -
+	55	0
-	0	38

All specimens were originally identified by use of the GenProbe MTB complex confirmation test.